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Development of an Efficient and Sensitive Magnetic Dispersive Solid-phase Extraction Technique for Preconcentration of Amphetamine and Methamphetamine Determined by High-performance Liquid Chromatography and Liquid Chromatography-Tandem Mass Spectrometry in Sports Supplements

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Sports and weight control supplements are tempting targets for adulteration. Amphetamines have been mainly prescribed as weight-loss drugs in the past decades. Amphetamines are also abused by athletes to improve their performance. To ensure the content of sports supplements, we propose a simple and straightforward magnetic dispersive solid-phase extraction method to extract amphetamine (AM) and methamphetamine (MET) from sports supplements. A few milligrams of magnetic graphene oxide ($GO@Fe_3O_4$) were added to the sample solution for microextraction assisted by shaking. Due to the benefit of dispersion and the high mass transfer rate of the sub-microscale adsorbent, the extraction equilibrium was achieved in a very short time (3 min). Moreover, thanks to the magnetic properties of adsorbent, the separation of the adsorbent from sample solution was easily achieved by an external magnetic field, which therefore simplified the sample pretreatment procedure. A high-performance liquid chromatography-ultraviolet (HPLC-UV) method with precision and accuracy better than 10% was proposed to detect AM and MET in the range of 300-1500 ng ml^{-1} and 500-2000 ng ml^{-1}, respectively. As a confirmatory analysis of determined AM and MET, an ion-spray LC-MS/MS method using multiple reaction monitoring (MRM) mode was applied. The LC-MS/MS method was linear in the range of 5-100 ng ml^{-1} and 1-200 ng ml^{-1} for AM and MET, respectively. Sixteen sports supplements; were scanned using the proposed method and AM and MET were confirmed in four out of sixteen samples.

KEYWORDS: Magnetic dispersive solid phase extraction, Amphetamines, Sports supplement, Adulteration, Mass spectroscopy

INTRODUCTION

In recent years, food supplements have attracted more attention worldwide because, in people's minds, they have fewer adverse effects than pharmaceuticals. As athletes and people use food supplements to control weight, enhance muscle size, and increase body energy, companies try to meet the required demands [1]. Defrauding sports supplements with different types of pharmaceutical products is comprised of various adulterants [2]. Adulterated products not only have a harmful effect on consumers' health but also trigger illegal activities. Studying how the content of supplements is determined by their label is an important task.

Amphetamine (AM) and methamphetamine (MET) are central nervous system (CNS) stimulants and increase brain performance. AM is a man-made compound, and its natural origin has rarely been reported [3]. Medications, including

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amphetamines, are used for several illnesses such as narcolepsy, obesity, and attention deficit/hyperactivity [4]. These drugs are included on the banned list by anti-doping agencies, but some consumers have misused them due to their synergic effects on exercise. Weight loss consistently ranks at the top of the list of demands among the world's people. İn the past decades, amphetamines were used as appetite suppressive agents, but their overall cardiovascular side effects pulled back from the market in 1997 [5]. Because of the weight loss effect of amphetamines, some food supplement producers add these drugs into their products to reduce their impact. Although amphetamines work well in decreasing weight consumers are not aware of the side effects of amphetamines [6]. Like most stimulants, amphetamines may cause extensive feelings of euphoria, can be addictive and are used for purposes other than medication [7].

Many dietary supplements have complex mixtures comprising proteins, fat, carbohydrates, *etc*., so a sample treatment step is needed to determine the target analyte inside such a mixed matrix. Applying appropriate preconcentration methods for enhancing the detection limit as well as providing a clean extract is a suitable solution when using fewer sensitive detectors such as UV.

Liquid-liquid extraction (LLE) and solid-phase extraction (SPE) is two of the well-known methods applied in the extraction domain. Compared to LLE, SPE offers more advantages, such as less time and lower usage of organic solvents [8-12]. The sorbent is an important part of SPE. In traditional SPE, which used cartridges or discs, the liquid phase might block the column; hence, they are typically used for single extraction and discarded [13]. Recently, magnetic dispersed solid-phase extraction (MDSPE) is known as an interesting microextraction approach [14]. Among the different sorbents, carbon and its diverse derivatives show some surprising features such as wide surface area (2630 m^2 g^{-1}), ability to form π-π situation, stability, and modification with different functional groups [15,16].

In the present work, we intend to use a simple extraction method, MDSPE, to determine the presence of AM and MET in sports supplements available on the market. We use a straightforward HPLC-UV set up to quantify the AM and MET. For further confirmation of the analysis, a reliable LC/MS-MS approach was arranged as proof of suspect samples. Different brands of commonly used sports supplements (16 brands) were bought from the local market and were tested under the proposed MDSPE-LC/MS-MS method.

MATERIALS AND METHODS

Regents and Materials

Standard materials of ($C_9H_{13}N{\cdot}HCl$) and ($C_{10}H_{15}N{\cdot}HCl$) both with purity > 99% were provided by Sigma. Graphite powder, KH_2PO_4, $FeCl_2{\cdot}4H_2O$, $FeCl_3{\cdot}6H_2O$, KOH, NaOH, NH_3 (25%), H_2SO_4, H_2O_2, $KMnO_4$ and HCl were prepared from Merck Chemicals (Darmstadt, Germany). Acetonitrile, acetone and methanol (all in HPLC grade) were purchased from Duksan Co. Ltd. (Ansan, South Korea). Double distilled water was kindly donated by Shahid Ghazi Pharmaceutical Co. (Tabriz, Iran). AM and MET standard solutions were made in methanol (1000 μg ml^{-1}). Needed concentrations were made from stock solution by dilution with methanol. All solutions were prepared freshly and the stock solution was put at 4 °C until use.

Apparatus and Chromatographic Condition

HPLC-UV instrumental works were accomplished on an Agilent (Germany) system coupled with a UV detection system and an injector with a 20 μl loop. A C18 column (10 μm particle diameter, 4.6 mm i.d. × 15 cm) (Agilent, Germany) was selected for the separation of analytes and the temperature was set at room temperature. The mobile phase composition was acetonitrile/phosphate buffer solution (10 mM) at a ratio of 15/85 (V/V) and the final pH of 3.5 were used in the isocratic mode at a flow rate of 1 ml min^{-1}. The UV detector wavelength was set at 220 nm.

LC/MS-MS Apparatus, MS Parameters, and Chromatographic Conditions

The mobile phase system on the HPLC was an isocratic mode consisting of 25 mM aqueous ammonium formate and acetonitrile (18:82, v/v) adjusted to pH 3 with formic acid. The flow rate was set at 0.25 ml min^{-1} and the column temperature was fixed at room temperature. Atlantis HILIC Silica column (150 mm × 3 μm i.d., 3 μm particle size, Milford, MA) was used for separation.

A triple quadrupole tandem mass spectrometer in positive mode was accompanied by HPLC system as a detector.

Appropriate MS/MS parameters for proper AM and MET analysis were settled by direct infusion of 1 µg ml^{-1} of AM and MET into the ESI source. The analytes' positive ions were fragmented in the collision cell by nitrogen as the collision gas. The protonated molecular ion of m/z 135.8 and m/z 149.9 corresponds to the molecular ion [M+H]+ of AM and MET, respectively. The MRM transitions of m/z 135.8 > 90.85 and m/z 149.9 > 90.85 were recorded for AM and MET at 200 ms per transition dwell time. The ESI instrumental settings were as follows: probe temperature, 500 °C; ion gas 1, 50 psi; ion gas 2, 50 psi; ion spray potential, 5000 V; collision gas, 8 psi; curtain gas, 28 V; and collision cell exit potential, 15 eV.

Synthesis of Magnetic Graphene Oxide (GO@Fe_3O_4)

Graphene oxide (GO) was prepared by improved Hummer's technique which is known as a green methodology [17,18]. Accurately weighted graphite powder (0.5 g) and concentrated H_2SO_4 (12 ml) were added into a reaction flask in an ice bath with stirring. Then, 1.5 g of $KMnO_4$ was gradually added into the reaction flask and then put in an oil bath shacking at 35 ºC. When the color of the solution was turned to light brown (after around 30 min), 15 ml distilled water was added to the mixture, and it was shacked for 30 min at 90 ºC. Then, H_2O_2 solution (1 ml of a 30% solution) was added to the reaction as a terminator agent, changing the color of the solution to dark yellow. The resultant mixture was put in a centrifuge, the supernatant was discarded, and solid phase was washed repeatedly with distilled water to eliminate the pollutants. The resulting GO particles were dried at 70 ºC.

İn order to prepare the GO@Fe_3O_4 adsorbent a chemical co-precipitation route was selected [19]. First, GO particles were made in 3 mg ml^{-1} in water solution by shaking in an ultrasonic bath for 30 min. Then, 10 ml solution of 100 mg of $FeCl_3.6H_2O$ and 45 mg of $FeCl_2.4H_2O$ were gradually poured into the reaction flask under the nitrogen stream. The reaction mixture was stirred vigorously for 30 min. The pH of the solution was adjusted with an ammonia solution (25%) until the pH reached around 11, and the mixture was stirred for 2 h. The temperature of the reaction was elevated to 80 ºC and remained for 2 h and then the temperature was cooled back to the room temperature. The resultant solid phase was collected by an external magnetic field and then washed several times with distilled water and dried at 70 ºC. The confirmation of GO@Fe_3O_4 was recorded in our previous work [14].

Magnetic Dispersive Solid Phase Extraction (MDSPE) Procedure

Around 0.1 g of blank sports supplement sample was finely grounded and dissolved in 5 mL methanol adjusted at pH 10 and centrifuged. The clear supernatant was put in another test tube and the following method was applied; a proper amount of 10 mg GO@Fe_3O_4 was weighted and was added to 5 ml of the sample and was shaken for 3 min. Solid phase was collected using a magnet and supernatant was removed. İn order to separate the analytes from the adsorbent, 300 µl of elution solvent (acetone) was added to particles and sonicated in 3 min using a sonicator. Then the solid particles were easily separated with a magnetic field and acetone-containing analytes were injected into the analytical device.

Sample Preparation

The samples were purchased from a drugstore (Etminan, Tabriz). These samples were mainly in three forms: capsules, tablets, and powder. In the case of capsules, the husks were discarded and the powder was taken off. All samples were homogenized into fine powders, and 0.1 g of each powdered sample was added into a 5 ml test tube containing methanol, and the supernatant was subjected to treatment by the MDSPE process after centrifuging.

RESULTS

Characterization of GO and GO@Fe_3O_4

Fourier transform infrared (FT-IR) spectrometry (Tensor 27; Bruker; Germany) was applied at 400-4000 cm^{-1} to characterize the synthesized sorbents. The FT-IR spectrum (Fig. 1) of bare GO shows peaks at 1732, 1622, 1395, and 1049 cm^{-1}, which correspond to the C=O stretching of COOH groups, C=C stretching vibration, C-OH stretching vibration, and C-O vibrations from alkoxy groups, respectively. The broad peaks at 3430 and 1261 cm^{-1} are related to the stretching vibration of the O-H and CO-H bonds, respectively. This information about the appeared peaks confirms the successful synthesis of GO by Hummer's

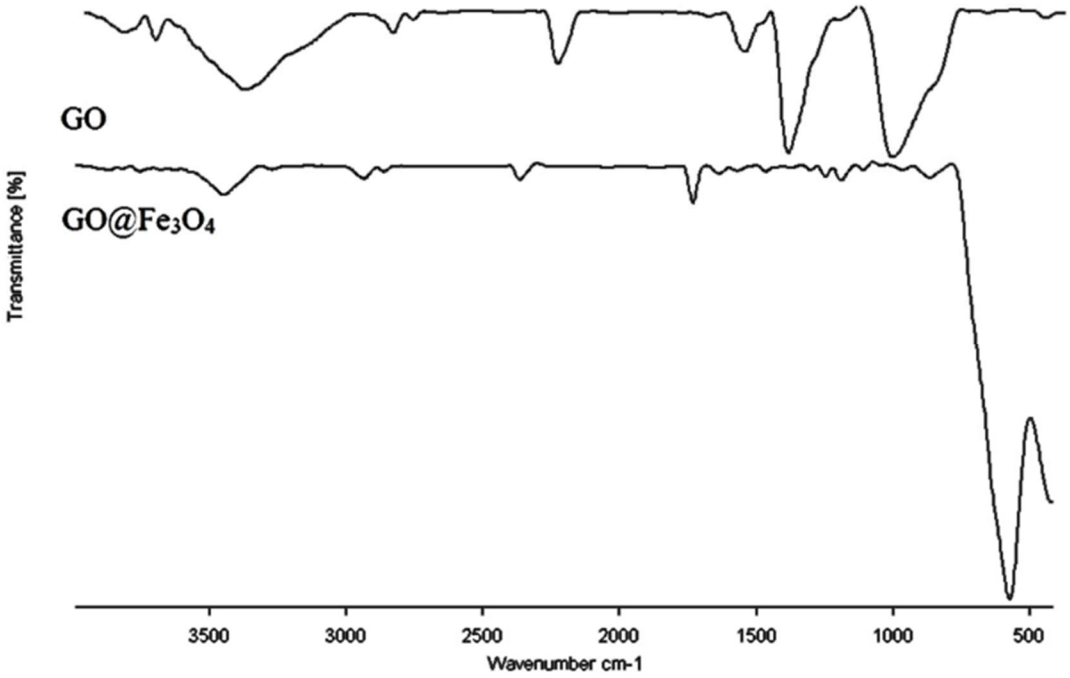

Fig. 1. Fourier transform infrared spectroscopy spectrums of GO and Go@Fe_3O_4 [14].

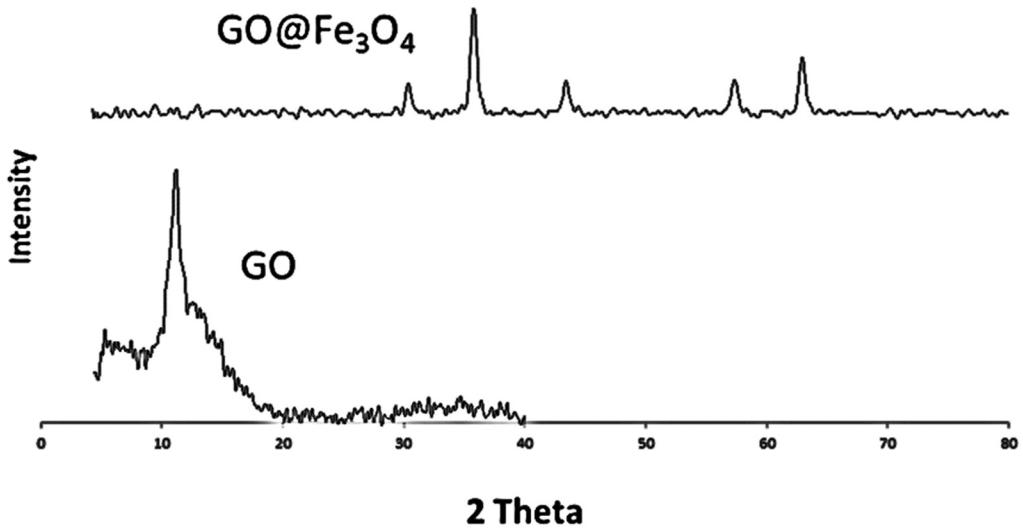

Fig. 2. X-ray diffraction patterns of GO and Go@Fe_3O_4 [14].

method. The attachment of Fe_3O_4 nanoparticles reinforced the GO on its surface and the success of the attachment was confirmed by IR as shown in Fig. 1. The explicit peak appeared at 564 cm^{-1}, related to the Fe-O band, confirming the presence of Fe_3O_4 nanoparticles in the GO@Fe_3O_4 structure.

The crystallographic structure of GO, and GO@Fe_3O_4 were confirmed by X-ray diffraction analysis (XRD) patterns and are shown in Fig. 2. GO shows a peak at $2\theta = 11.3°$, confirming that the distance between GO sheets is due to the presence of functional groups such as hydroxyl, epoxy, and carboxyl groups attached to inter-planner sheets from both

sides. As shown in Fig. 1, the reflections for intense peaks of (2 2 0), (3 1 1), (4 0 0), (4 2 2), (5 1 1), and (4 4 0) are indexed to plans at 2θ = 30.15, 36.27, 43.32, 53.89, 57.13, and 62.29°, respectively; these reflections are conducted to synthesize GO@Fe_3O_4 successfully.

Scanning electron microscope (SEM) analysis was applied to elucidate the morphology of the adsorbent as shown in Fig. 3. GO shows a winkle and layered appearance. Compared with unmodified GO sheets, the uniform decoration of magnetic particles are observed on the surface of GO in the GO@Fe_3O_4 composite.

The magnetic materials utilized in this study exhibited a superparamagnetic behavior. Figure 4 shows a typical hysteresis loop of pure Fe_3O_4 and GO@Fe_3O_4. The saturated

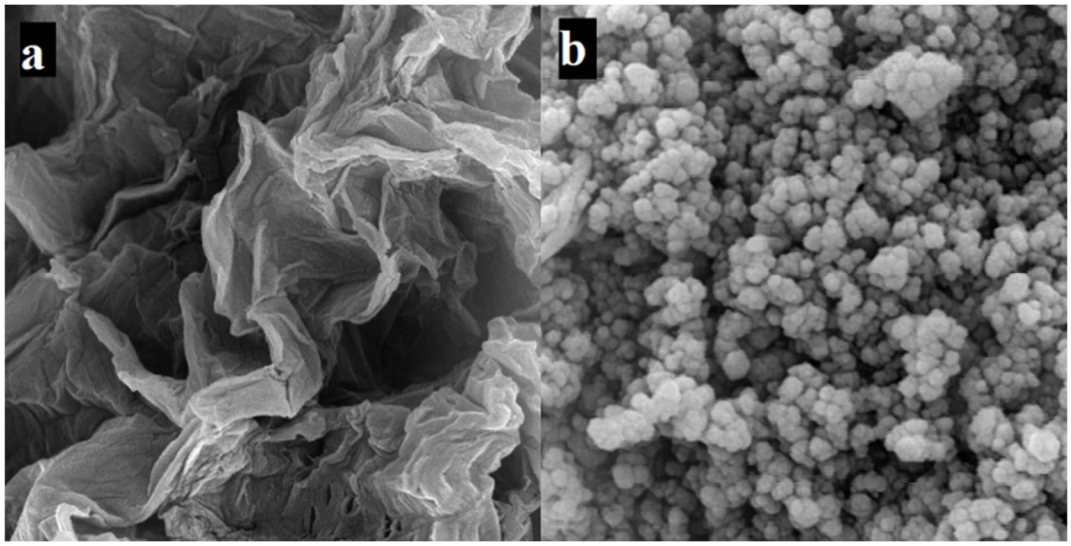

Fig. 3. Scanning electron microscopy images (a) of graphene oxide (GO), and (b) Go@Fe_3O_4.

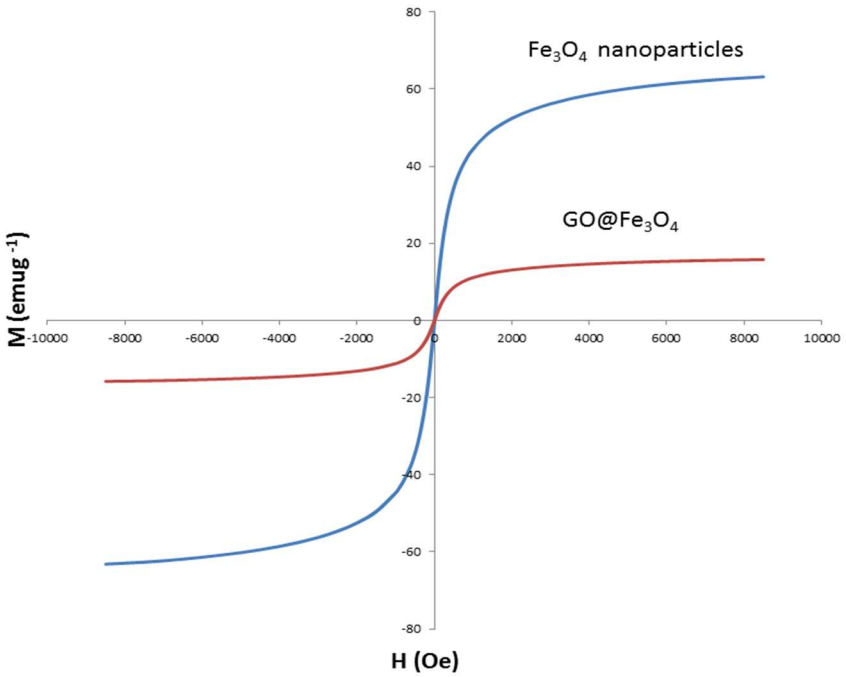

Fig. 4. Magnetization hysteresis loops of GO and Go@Fe_3O_4 [14].

magnetization M_S of pure magnetic nanoparticles was 48.14 emu g^{-1}, while in the saturation magnetization curve it reached in the lower amount due to the presence of GO layers.

Optimization of Important Parameters Involved in the Magnetic Dispersive Solid Phase Extraction

The well loaded the intended analytes on the MNPs are of prime. However, co-existing materials and other interference are present in the sample matrix and can compete with target analytes in the extraction process. To assess this, important parameters involved in the MDSPE process were carefully investigated. These factors are the kind and amount of adsorbent, the pH of the sample solution, adsorption time, type and volume of the eluting solvent, desorption time, pH, and sample volume. These terms were optimized one by one by the spiking of 0.1 µg ml^{-1} of AM and MET in sample media.

First, to have maximum extraction recovery of AM and MET, two types of adsorbents were checked, GO@Fe_3O_4 and Fe_3O_4. Based on observations, the extraction capacity of GO@Fe_3O_4 was significantly higher than Fe_3O_4. The GO@Fe_3O_4 adsorbent contains plenty of oxygen-containing groups, and they help the excellent dispersion of this adsorbent versus unmodified Fe_3O_4. Therefore, it was decided that MNPs were used as an adsorbent in this investigation. The number of MNPs is an effective factor and is proportional to how many analytes could be loaded. According to Fig. 5a, different dosages of adsorbent *i.e.*, 10, 20, 30, and 40 mg were tried and findings show that 10 mg of adsorbent was appropriate for maximum extraction of target analytes. Finally, 10 mg of the MNPs opted for all the following processes. Compared to traditional SPE, MDSPE provides a larger surface-to-volume ratio; therefore, more assessable cites are available for analyzing loading.

The time interval between the analytes being extracted until they are isolated from the adsorbent is defined as the time of extraction. To evaluate this parameter, different times from 3 to 10 min were tested, and according to Fig. 5b, in 3 min, almost the maximum amounts of analytes were extracted. Exceeding the time of extraction by more than 3 min did not show any positive effect on the extraction recovery. The Langmuir adsorption capacities of selected materials (in mg g^{-1}) of GO@Fe_3O_4 were calculated and are 98.08 (mg g^{-1}) and 103.09 (mg g^{-1}) for AM and MET, respectively. The Langmuir adsorption capacities of Fe_3O_4 are 16.86 (mg g^{-1}) and 15.90 (mg g^{-1}) for AM and MET, respectively. After the analytes are extracted from the sample medium, they should be eluted with an appropriate solvent. Acetonitrile, acetone, and methanol are routine and available solvents tested as desorption solvents in the MDSPE process. To do this process, a set of examinations were arranged using 800 µl of each desorption solvent, and the results were recorded. Observations showed that desorption with acetone (93.66 ± 3.51%) is better in comparison to acetonitrile (64.02 ± 1.50%) and methanol (51.00 ± 2.01%). Thus, we chose acetone among these solvents for the subsequent experiments. The volume of the extraction solvent influences the performance of the extraction process. The acetone volumes were changed to 300-900 µl to record the results. Findings in Fig. 5c show that 300 µl of acetone has adequate ability in the desorption of analytes. By exceeding the amount of acetone, the recoveries were decreased, maybe because of the dilution effect. To complete the desorption process, enough time must be given for the analytes to be eluted from the adsorbent. At this stage, the time of desorption was checked in the range of 3 to 10 min and data showed that in 5 min, the process was completed.

Adjusting the pH of the sample solution directly affects the ionic state of the analytes, adsorbent, and other co-existing materials that may be present in the sample solution. Therefore, the sample solution pH was set in the range of 8-12 and as shown in Fig. 5d, the analytical response is its maximum amount when the pH is set at 10. In addition, according to Zeta potential analysis (-41 mV), negative ion density is high enough to prevent accumulation between adsorbent sheets [20].

The sample volume is an important parameter to provide enough environment to disperse the adsorbent. The evaluation of this parameter was scanned at 2 to 10 ml. As presented in Fig. 5e, the recoveries were good in 5 ml. By increasing the sample volume up to 10 ml, the recoveries may get worse because of the adsorbent's decreasing dispersibility. Therefore, samples were prepared in 5 ml of methanol.

Method Validation

While the HPLC-UV method is more cost-effective than

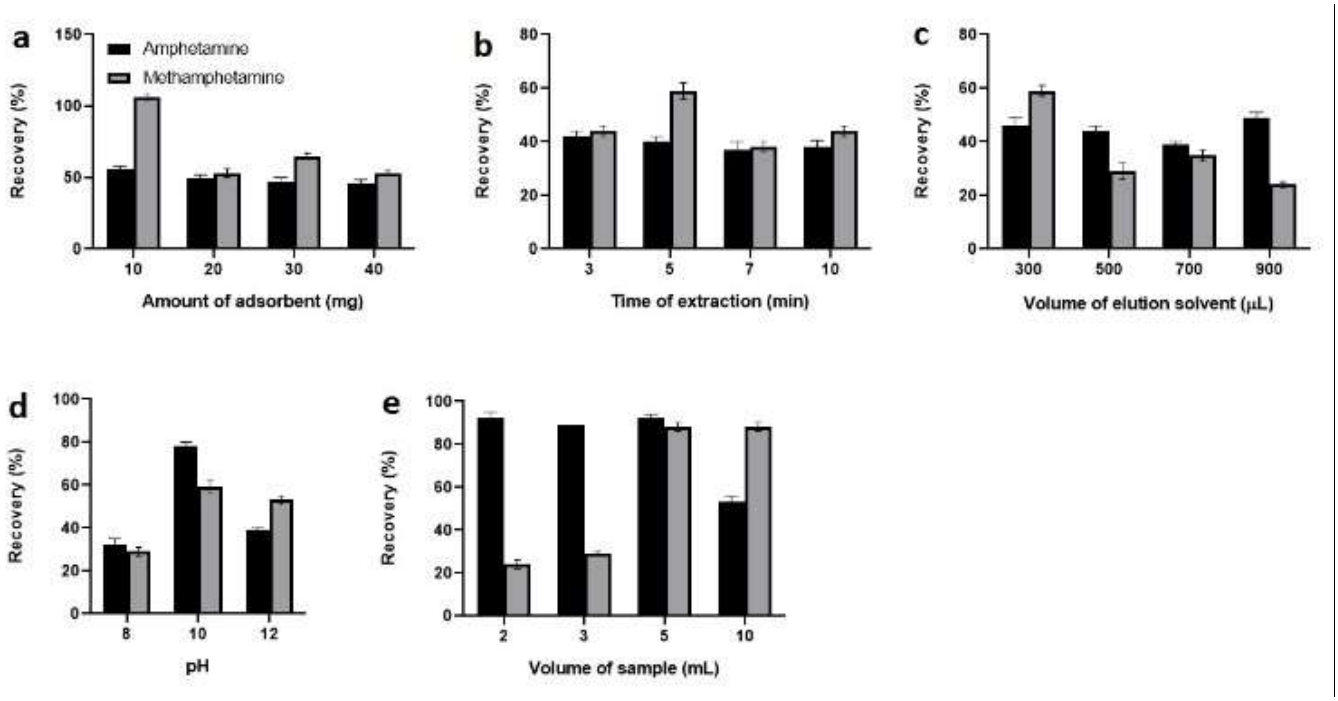

Fig. 5. Effect of (a) various amounts of GO@Fe_3O_4 sorbents (mg), (b) and contact time on extraction efficiency, (c) volume of elution solvent, (d) pH, and (e) volume of the sample are given. Mobile phase system on the HPLC was an isocratic mode consisting of 25 mM aqueous ammonium formate and acetonitrile (18:82, v/v) adjusted to pH 3 with formic acid. The flow rate was set at 0.25 ml min^{-1} and the column temperature was fixed at room temperature. Atlantis HILIC Silica column (150 mm × 3 μm i.d., 3 μm particle size, Milford, MA) was used for separation. The protonated molecular ion of m/z 135.8 and m/z 149.9 corresponds to the molecular ion [M+H]+ of AM and MET, respectively. The MRM transitions of m/z 135.8 > 90.85 and m/z 149.9 > 90.85 were recorded for AM and MET at a dwell time of 200 ms per transition. The ESI instrumental settings were as follows: probe temperature, 500 °C; ion gas 1, 50 psi; ion gas 2, 50 psi; ion spray potential, 5000 V; collision gas, 8 psi; curtain gas, 28 V; and collision cell exit potential, 15 eV.

sophisticated methods, in the case of complex matrices and co-exciting species, which may be present along with the analyte, a confirmatory method seems to be necessary. The LC-MS/MS system is a sensitive method that accurately confirms the analytes' peaks [21]. However, applying the HPLC-UV method to quantifying AM and MET has a shortcoming in confirming analytes' peaks. Some co-existing materials may have a retention time matching that of AM and MET. So, we decided to develop an extra LC-MS/MS method (described in the experimental section) to obtain proof of analytes' peaks. The chromatograms of the/MS-MS method. Figure 6 shows chromatograms of AM and MET obtained from LC/MS-MS.

The need for such a method was essential since several compounds shared the same retention time pattern in HPLC-UV analysis and were not confirmed by the UV used in the original method. AM and MET are isolated with MDSPE and quantified using a calibration plot. The concentration levels needed for constructing the calibration curve were made in a matrix-matched way to ensure that the matrix effect is considered in the calculations. Consequently, we prepared the calibration standards by adding AM and MET working standard solutions into blank matrices. Table 1 shows details of the mathematical equation in the linear range. The limits of detection (LOD) and limit of quantification (LOQ), were defined as a concentration producing a signal 3 and 10 times the standard deviation of the blank, respectively. The LODs ranged from 15 to 70 ng ml^{-1}, while the LODs included from 0.1 to 0.5 ng ml^{-1} for HPLC/UV and LC-MS/MS, respectively. The enrichment factor was calculated to be 10.

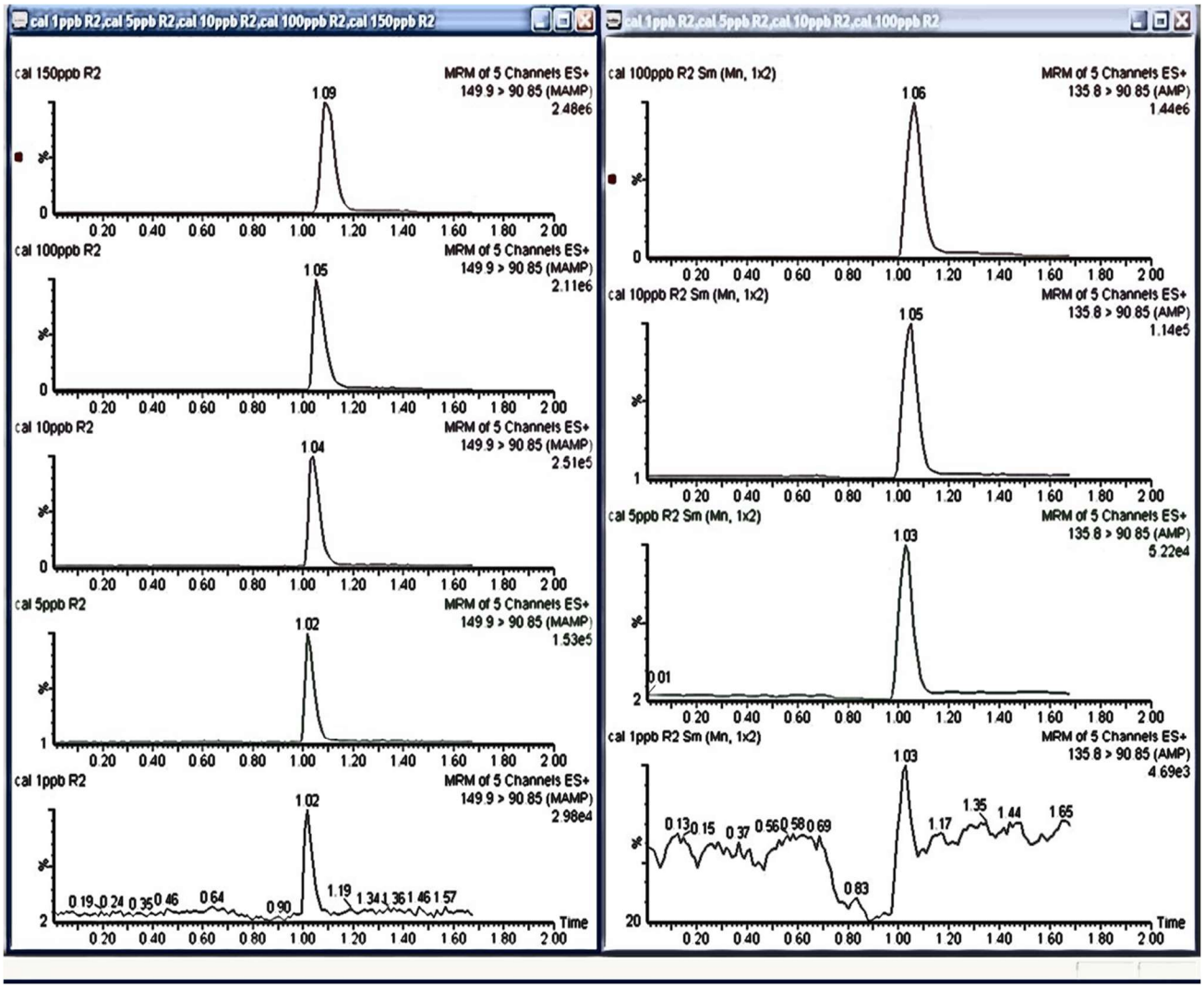

Fig. 6. Chromatograms of amphetamine and methamphetamine. LC/MS-MS condition was stated in Fig. 5. The experimental condition for magnetic dispersive solid phase extraction (MDSPE) is as follows; the amount of adsorbent: 10 mg GO@Fe_3O_4, pH 10, extraction time: 3 min, of elution solvent volume: 300 µl of acetone, elution time: 3 min.

Table 1. Some Analytical Characteristics for Magnetic Dispersive Solid Phase Extraction of Amphetamine and Methamphetamine

Analyte	Method	Concentration range (ng ml^{-1})	Linearity (r^2)	LOD (ng ml^{-1})[b]	LOQ (ng ml^{-1})[c]	RSD (%)[d]
Amphetamine	HPLC/UV	300-1500	0.991	70	210	6.84
Methamphetamine	HPLC/UV	500-2000	0.992	15	47	7.54
Amphetamine	LC/MS-MS	5-100	0.996	0.1	0.5	6.50
Methamphetamine	LC/MS-MS	1-200	0.996	0.5	0.7	6.92

[a]Linearity is described by the correlation coefficient for the calibration curve. [b]Limit of Detection (LOD) S/N = 3. [C]Limit of Quantification (LOQ) S/N = 10. [d]Relative Standard Deviation.

Repeatability and accuracies experimented on two occasions; one day and between days. As evaluated in Table 2, the average RSD% was less than 10%. Therefore, it is proof of processing reliability. MDSPE purified the matrix from unknown and co-existing materials well, and the blank matrix was very clean. The chromatograms for the blank and spiked samples obtained from HPLC-UV after MDSPE are shown in Figs. 1S and 2S.

Real Samples

The proposed method was tried on sixteen different dietary supplements provided by local markets, and they were in capsule, tablet, or powder form. The MDSPE procedure was applied to all samples described in the experimental part and analyzed with LC/MS-MS. The information on the samples is provided in Table 3. As the matrices analyzed are in different matrices, the standard

Table 2. Results of Method Validation of Magnetic Dispersive Solid Phase Extraction

Concentration ($\mu g\ ml^{-1}$)		Intra-day (n = 3)		Inter-day (n = 3)	
		Precision (RSD*)	Accuracy (bias)	Precision (RSD)	Accuracy (bias)
Amphetamine	0.30	9.89	-0.82	6.50	+11.58
	0.50	13.14	-8.97	5.63	+2.10
	1.50	4.26	-2.35	6.43	+5.18
Methamphetamine	0.30	1.24	+0.54	3.61	+15.07
	0.50	14.28	+1.02	5.23	+6.57
	1.50	6.62	+1.98	3.31	-7.41

Table 3. Concentrations ($ng\ ml^{-1}$) of Amphetamine and Methamphetamine in Dietary Products

	LC/MS-MS		HPLC-UV	
Sample	AM (SD)	MET (SD)	AM (SD)	MET (SD)
1	ND	1.0 (0.2)	ND	ND
2	ND	ND	ND	ND
3	ND	ND	ND	ND
4	ND	1.8	ND	ND
5	ND	ND	ND	ND
6	ND	ND	ND	ND
7	ND	ND	ND	ND
8	ND	ND	ND	ND
9	ND	ND	ND	ND
10	ND	ND	ND	ND
11	ND	ND	ND	ND
12	ND	ND	ND	ND
13	32 (0.8)	700 (4.0)	ND	675 (2.9)
14	2.5	ND	ND	ND
15	ND	ND	ND	ND
16	ND	ND	ND	ND

Table 4. Some Reported Methods for Analysis of Amphetamine in Biological Samples

Method	Sample type	Sample preparation	Linear range	Ref.
UPLC/SRM-MS[a]	EBC	SPE[b]	-	[22]
LC/MS-MS	EBC	-	10-10,000 pg/filter (0.2-20 ng ml^{-1})	[23]
LC/MS-MS	EBC	Protein precipitation	10-9000 pg/filter	[24]
GC/MS	Urine	LLE[c]	125-1000 ng ml^{-1}	[25]
GC/FID[d]	Urine	HS-SPME[e]	125-3742 ng ml^{-1}	[26]
GC/MS	Hair	LLE	0.13-0.73 mg kg^{-1}	[27]
LC/MS-MS	Hair	LLE	0.050-50 ng mg^{-1}	[28]
LC/MS-MS	Hair	SPE[e]	0.050-4 ng mg^{-1}	[29]
GC/MS	Urine	SPE	7.81-5000 ng ml^{-1}	[30]
GC/MS	Urine	UA-LDS-DLLME[f]	0.15-10 µg ml^{-1}	[31]
GC/MS	Whole blood	SPE	0.1-50 ng ml^{-1}	[32]
	Urine	SPE	10-500 ng ml^{-1}	[33]
GC/MS	Urine	LLE	0.5-5.0 µg ml^{-1}	[34]
GC/MS	Serum	LLE	20-800 ng ml^{-1}	[35]
LC/MS-MS	Blood	SPE	2.5-400 µg l^{-1}	[36]
	Urine		25-1000 µg l^{-1}	
CSEI-sweeping[g]	Hair	LLE	0.05-20 ng ml^{-1}	[37]
MEKC/UV[h]	Blood	-	LOD: 1.2 µg ml^{-1}	[38]
CE/DAD	Whole blood	LLE	20-500 µg ml^{-1}	[39]
MEKC/UV	Urine	SPE	LOD: 100 ng ml^{-1}	[40]
CE/DAD	Urine	LLE	0.156-40 µg ml^{-1}	[41]
CE/UV-FASS[i]	Urine	SPE	0.1-5 mg l^{-1}	[42]
CEC/UV	Urine	SPE	100-1200 ng ml^{-1}	[43]
CE/LIF	Urine	SPE	0.5-100 ng ml^{-1}	[44]
CE/MS-FASS	Hair	LLE	0.025-5 ng mg^{-1}	[45]
HPLC/MS-MS	Dietary supplements	LLE	25-2000 ng ml^{-1}	[3]
CE/MS-MS	Medicine/ dietary supplements	LLE	1.0-200 µg l^{-1}	[46]
LC/MS-MS	Dietary supplements	MDSPE[j]	1-200 ng ml^{-1}	This work
HPLC-UV	Dietary supplements	MDSPE	300-200 ng ml^{-1}	This work

[a]Ultra-performance liquid chromatography-selected reaction monitoring mass spectroscopy. [b]Solid phase extraction. [c]Liquid-liquid extraction. [d]Gas chromatography flame-ionization detection. [e]Headspace solid-phase microextraction. [f]Ultrasound-assisted low-density solvent dispersive liquid-liquid microextraction. [g]Cation-selective exhaustive injection. [h]Micellar electrokinetic capillary chromatography. [i]Capillary electrophoresis ultra violet field amplified sample stacking. [j]Magnetic dispersive solid phase extraction.

addition method was applied to calculate the recovery. The extraction recovery was obtained for each real sample using 0.1 µg ml^{-1} of analytes standard and was calculated from 85% to 112% for powder forms and from 80% to 106% for tablet and capsule forms using LC-MS/MS.

DISCUSSION

Table 4 summarizes various methods reported in determining AM and MET with various analytical techniques. Proposed method has a wide linear range

(1-200 ng ml^{-1}) using LC/MS-MS system. The extraction process of our method takes a short time and extraction efficiency is very high. The proposed MDSPE methodology gives a clean extract, as the chromatogram of the blank is very smooth. All these observations resulted that the proposed method, namely MDSPE/LC/MS-MS is sensitive and quick for the preconcentration and quantification of AM and MET as adulterants in the food matrix. This method overwhelmed the difficulties of the traditional SPE approach, such as several conditioning processes (loading, washing, and elution). Instead, these steps were performed in less than 10 min and used only 300 µ acetone.

CONCLUSİONS

Unfortunately, the adverse effects of some illegal additives in a few dietary supplements come at a high price for consumer health and safety. This is why researchers have focused on finding and controlling substances and adulterants that can be lurking in some sports and weight management products. In this project, the assay of AM and MET in sports supplements was tracked by MDSPE/LC-MS-MS. In summary, we proposed a simple MDSPE method for the extraction of AM and MET with GO@Fe_3O_4. Benefiting from the shaker assisted dispersion and the sub-microscale structure of the adsorbent, the extraction equilibrium can be accomplished within 3 min. The MDSPE was demonstrated to be rapid, high-throughput, easy-operation (without centrifugation), cost-effective and solvent-saving. The excellent performance in cleaning up the supplements matrix suggested that the miniaturized SPE format was effective for dealing with complicated food samples. Furthermore, the MDSPE approach may provide a general and promising method in the purification of other complex samples in microscale sample preparation field. This methodology is accurate and precise for routine AM and MET adulteration assays in food control laboratories.

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